SOME FACTORS CONTROLLING THE INGESTION OF CARBOHYDRATES BY THE BLOWFLY[1]

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Diet selection and preference are commonly evaluated in terms of quantity of food consumed; however, measurements of intake alone give little information concerning the degree to which different factors participate in the regulation of ingestion. It is clear in the case of insects that a sequential contribution by various stimuli governs the finding of food, the initiation of biting or sampling, the continuance of feeding, and the termination of feeding. It is believed by some (*e.g.*, Dethier, 1953; Fraenkel, 1953) that stimuli which initiate sampling and which drive continued feeding are neither necessarily nor invariably correlated with nutritional values. Other workers, notably Kennedy (1953), believe that there is an important causal relationship between stimulating and nutritional characteristics. The present study is intended as a step toward the ultimate clarification of this problem.

Carbohydrates were chosen as test compounds because they do not stimulate the olfactory sense and because they represent all possible combinations of stimulating effectiveness and nutritional value. There are sugars which are stimulating but non-nutritional, stimulating and nutritional, non-stimulating but nutritional, and non-stimulating and non-nutritional. Sugars representing these categories were employed in the following experiments: (1) preference-aversion tests in which were recorded the volumes imbibed by flies given a choice between sugar and water or between one sugar and another; (2) individual feeding tests in which volume intake was measured in the absence of a choice situation; (3) tests of the sensitivity of the different chemoreceptor systems to stimulation; (4) measurements of the volume intake of mixed solutions; (5) longevity tests to ascertain the nutritional value of the various sugars at different concentration levels.

Materials and Methods

Preference-aversion tests were conducted according to the procedure of Dethier and Rhoades (1954). In essence, the tests consisted of presenting groups of twenty flies, which had been enclosed in one-quart mason jars, with the choice of drinking from either or both of two J-shaped volumetric pipettes. The mean per capita fluid intake per twenty-four hours was calculated from the total volume of fluid taken from each pipette over a four-day period. In two-choice situations of this sort the intake of sugar can be compared with that of water or of any other sugar or sugar mixture.

[1] This investigation was supported by a grant from the National Science Foundation.

In order to ascertain the number of visits which were made to each pipette and the duration of each visit, the original apparatus was modified as follows. A silver wire was inserted into each pipette in such a way as to extend the entire distance from the large opening to a point just one millimeter short of the capillary orifice. Silver-conducting paint (DuPont Silver 4916) was then brushed in a thin line from a point near the large orifice to a point one millimeter short of the capillary orifice; here the painted line was extended around the circumference of the pipette so that a fly had to stand on the paint in order to drink. To the painted line near the large opening was soldered a silver wire. This wire and the wire from inside the pipette were each extended to the terminals of a Brush BL907 amplifier which in turn was connected to a BL202 recording instrument. Since the entire apparatus was intentionally unshielded, the two wires acted as antennae which picked up 60 cycle current from lights and various motors operating in the laboratory. Whenever a fly attempted to drink from a pipette, it closed the circuit between the conducting

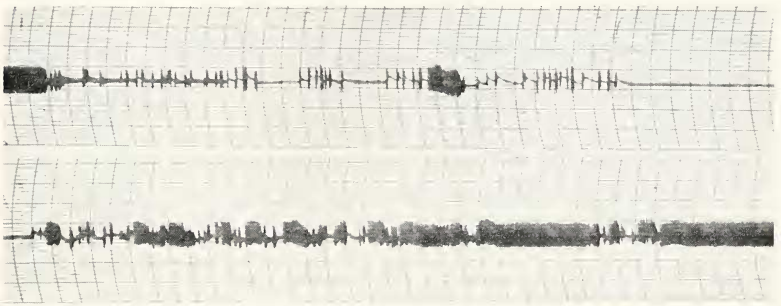

FIGURE 1. Typical example of automatically recorded periods of feeding. The thin line represents periods of feeding. Note that the fly has taken one long drink beginning at the upper right and continuing at the lower left. During the remainder of the time only brief samples were taken. Each curved line represents 5 seconds.

paint on which it was standing and the fluid and wire within the pipette. Since the 60 cycle current was then shorted out, the period of drinking appeared on the record as a straight line instead of alternating impulses (Fig. 1). The authenticity of records obtained by this method was confirmed by visual observation. At the same time the identity of the drinker was noted.

Finally, the fluid intake of individual flies was measured by direct analyses of sugar. For these measurements the flies were fed 24 ± 2 hours before testing on 0.1 *M* sucrose and then received neither food nor water until the experimental ingestion. At this time the flies were mounted on waxed sticks and individually fed on the test solutions. Some arbitrary criterion of repletion was necessary since a fly will continue alternately to extend and retract its proboscis almost indefinitely on some sugars, all the while taking small additional amounts. Repletion, therefore, was defined by the period of vigorous proboscis extension and active uptake. Usually a fly would feed continuously and actively for an initial prolonged period and then perhaps for an additional shorter period when its labellar hairs were brought

into contact with the solution. This period of active feeding was usually rather sharply delineated, as indicated by the agreement of duplicate determinations on different groups of flies treated similarly. The standard deviation of replicate determinations of volume intake ranged between 0.377 μl for 1 *M* sucrose and 1.34 μl for 1 *M* fucose.

The determination of quantity ingested was accomplished by a sensitive spectrophotometric reaction for carbohydrates employing anthrone in concentrated sulfuric acid (Dimler *et al.*, 1952). For each determination the abdomens of 5–20 flies were ground, immediately after feeding, in 10 ml. of 5% trichloroacetic acid. The crop and intestine, which contain the ingested sugar, are located entirely in the abdomen after feeding. Equally large groups of flies similarly treated, but fed nothing, served as controls. After centrifugation, aliquots of the supernatant were diluted appropriately to produce concentrations from 30 to 200 μg. sugar per ml.

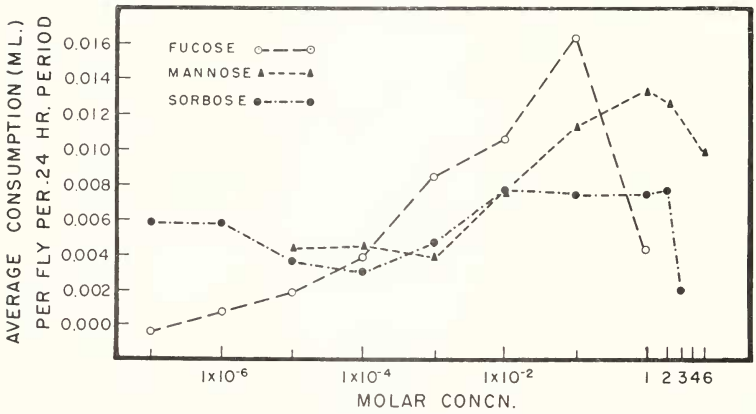

FIGURE 2. Preference-aversion curves for fucose, sorbose, and mannose.

One-ml. samples of these final dilutions were employed for the anthrone reaction and were compared spectrophotometrically with standards of the several sugars fed. The amount of sugar in the fed groups in excess of that in the unfed controls was directly convertible to volume since the concentrations of the solutions employed for feeding were known precisely. Longevity was measured with the same experimental set-up employed for preference-aversion testing.

PREFERENCE-AVERSION CURVES

The volume intake, as compared with water, was measured for each of the following sugars over the concentration range 1×10^{-7} *M* to saturation: fucose, sorbose, mannose. The results are summarized in Table I and Figure 2.

Fucose is a methyl pentose which is rather effectively stimulating for the tarsal chemoreceptors (median acceptance threshold = 0.087 *M*) but not utilized by the

TABLE I

Amount of solution consumed (ml./fly/24 hrs.) when sugar is paired with water

Molar concen.	Fucose	Water	Level (%) of significance of difference
1.0	0.0042	0.0035	—
0.1	0.0161	0.0012	0.1
0.01	0 0102	0.0031	0.1
0.001	0.0081	0.0041	0.1
0.0001	0.0037	0.0022	1.0
0.00001	0.0017	0.0033	—
0.000001	0.0008	0.0007	—
0.0000001	0.0000	0.0019*	0.1
	sorbose		
3.0	0.0020	0.0021	
2.0	0.0075	0.0036	0.1
1.0	0.0074	0.0027	1.0
0.1	0.0072	0.0036	0.1
0.01	0.0076	0.0050	0.1
0.001	0.0045	0.0030	1.0
0.0001	0.0030	0.0029	—
0.00001	0.0031	0.0012	—
0.000001	0.0058	0.0043	—
0.0000001	0.0059	0.0074*	5
	mannose		
6.0	0.0097	0.0024	5
4.0	0.0106	0.0016	5
2.0	0.0124	0.0011	1.0
1.0	0.0132	0.0023	0.1
0.1	0.0111	0.0022	0.1
0.01	0.0074	0.0032	0.1
0.001	0.0036	0.0046*	5
0.0001	0.0043	0.0054*	5
	rhamnose		
1.0	0.0068	0.0004	1.0
0.1	0.0067	0.0005	1.0
	lactose		
1.0	0.0212	0.0159	1.0
0.1	0.0091	0.0012	1.0
	D-arabinose		
0.1	0.0122	0.0004	0.1
	L-arabinose		
0.1	0.0035	0.0036	—

* These values represent the concentration range where water is taken in significantly greater amounts than sugar.

blowfly *Phormia regina* (Hassett, Dethier and Gans, 1950). Sorbose, a hexose, also stimulates the tarsal chemoreceptors (threshold = 0.14 *M*) although it is not utilized. Mannose, a hexose, is extremely poor in stimulating power (tarsal threshold = 7.59 *M*) but is nutritionally highly effective.

The curves describing the ingestion of the three sugars are substantially similar to those obtained by Dethier and Rhoades (1954) with the nutritionally adequate sugars glucose and sucrose. In each case there is a low concentration at which the sugar is not distinguished from water so that equal amounts of solution are taken from each pipette. Then, as the concentration is increased, a point is reached where more sugar than water is imbibed. This point represents a difference threshold. It occurs at a lower concentration than the tarsal acceptance threshold obtained by standard procedures. As the concentration is further increased there is an increase in the volume of solution imbibed until a maximum intake is reached, after which there is a marked decrease. A cursory examination of the curves reveals no relation between the volume intake and either the nutritional value or the relative stimulating effectiveness. Of the three sugars, the maximum intake is greatest for fucose and least for sorbose. None is consumed in as great quantities as glucose or sucrose.

Another characteristic of these curves is an inversion at very low concentrations where water may be taken in preference to sugar. With fucose, sorbose, and mannose the inversion occurs at 1×10^{-7} *M*, 1×10^{-7} *M*, and $1 \times 10^{-3} - 1 \times 10^{-4}$ *M*, respectively. Bimodal preference-aversion relationships of sugars were first noted by Beck (1956) in studies of the larvae of the European corn borer (*Pyrausta nubilalis* Hbn.). A re-examination of the raw data of Dethier and Rhoades (1954) reveals similar relationships. The meaning of rejection at low concentrations is not at all clear.

Individual Intake

When measurements were made of the volume of different concentrations of sugars imbibed by a single fly at one feeding (Table II) and the values plotted as a function of the concentration, the resulting curves differed in several important respects from the customary preference-aversion curves (Fig. 3). With the exception of fucose and sucrose there was no evident tendency for intake to decrease at high concentrations. There was, however, a marked tendency for intake to reach a plateau. On the other hand, regardless of the procedure employed for measuring intake, the weight of sugar consumed increased throughout the entire concentration range. There is no indication that the flies regulate the quantitative intake of sugar.

In comparing individual feeding curves with preference-aversion curves based upon four days of feeding the further difference is noted that the volume intake, while approximately the same in both experiments at high concentrations, at low concentrations is much smaller when measured individually than when measured in a two-choice situation. The fact that one experiment involves a two-choice situation while the other involves no choice has no bearing on the results because Dethier and Rhoades (1954) have shown that intake is the same in one-choice and two-choice situations. It seems possible to explain the difference on the basis of gustatory thresholds and behavior as affected by feeding. Earlier work (*cf.* Dethier and Chadwick, 1948) indicated that feeding elevates taste thresholds, and it seems

reasonable to assume that the greater the ingestion of sugar the longer the taste threshold remains elevated (this assumption is borne out by experiments, soon to be published, on the determinants of taste threshold in *Phormia*). Furthermore, present data show that in general the volume ingested at a single feeding is a direct function of the stimulating effectiveness of the test solution. Hence, it might be expected that in preference-aversion experiments, after once feeding on 1.0 or 2.0 *M*

TABLE II

Amounts of various sugars ingested at a single feeding

Sugar	Molar concentration	Number of animals	Mg./fly	Ml./fly $\times 10^3$	Duration* (sec.)	Rate ml./sec. $\times 10^5$	Approximate viscosity (centipoises)
Sucrose	2.0	20	8.96	13.0	90	14	—
	1.0	30	4.78	13.9	47	30	—
	0.5	10	1.80	10.5	43	24	—
	0.25	10	0.440	7.05	36	20	—
Glucose	2.0	15	4.92	13.7	61	26	—
	1.0	35	2.27	12.6	44	30	—
	0.5	15	0.820	9.11	38	25	—
Mannose	4.0	15	6.49	9.02	51	18	—
	2.0	15	2.97	8.25	40	21	—
	1.0	10	1.12	6.20	38	16	—
	0.5	10	0.268	2.98	25	12	—
Fucose	1.0	50	0.843	5.14	30	20	—
	0.5	15	0.580	7.08	32	35	—
Lactose	1.0	15	0.903	2.82	18	18	—
Sorbose	3.0	10	1.93	3.58	—	—	—
	2.0	20	1.09	3.04	—	—	—
	1.0	10	0.168	0.934	—	—	—
	0.5	6	0.0481	0.534	—	—	—
Sucrose	1.0	10	4.62	13.5	54	25	2.75
Sucrose 1.0*M* in:							
Glycerol	2.2	10	5.20	15.2	60	25	4.29
	5.4	10	2.86	8.34	50	17	7.79
	8.7	4	2.20	6.45	55	12	48.5

* Duration times were recorded for fewer flies than were employed in ingestion determinations.

sugar, the fly would not respond to the solution again for some time when it is encountered; and, furthermore, that when again ingested the solution will be taken in far lesser quantities as a result of the partially elevated threshold. Moreover, the number of encounters with the sugar solution is markedly reduced with flies feeding on 1.0 or 2.0 *M* sugar, since they are almost completely inactive for some time after ingestion of a large sugar meal. When 0.1 or 0.01 *M* sugar solutions are employed for preference-aversion tests, the post-ingestion duration of threshold elevation, the

period of quiescence, and the interval during which response fails upon contact with the solution are all shortened relative to the higher concentrations. The frequency of feeding is thereby increased. Thus may be explained the discrepancy of a higher daily intake of 0.1 *M* than 1.0 *M* sucrose, although at a single feeding much more is taken of the higher concentration.

The action of the above factors is again seen when the raw data of the preference-aversion curves of Dethier and Rhoades (1954) are analyzed on a day-to-day basis. It was found that curves based solely on the first 24-hour intake were displaced to the right, that is, the maximum intake occurred at very high concentrations. For subsequent 24-hour periods the intake of high concentrations drops while that of low concentrations gradually increases (see Dethier and Rhoades, Fig. 2).

The expectation of more frequent feeding on 0.1 *M* than 1.0 *M* sucrose was confirmed by automatic recordings of preference-aversion behavior. During the first eighteen hours of recording, 791 drinks were taken from 0.1 *M* sucrose and only 236 from 1.0 *M*. During the same period there were in addition 1,336 tentative drinks or taste samples of 0.1 *M* as compared with 898 of 1.0 *M*. The duration of drinking was approximately the same with each concentration; however, the volume imbibed per drink of 1.0 *M* was slightly more than twice that of 0.1 *M*. The rate of intake was, therefore, greater in the case of 1.0 *M*. It was also noteworthy that over the entire 18-hour period there was no marked decrease in the number of drinks of 0.1 *M* per hour, but the number of drinks of 1.0 *M* per hour had decreased by 80% at the end of 12 hours. The number had reached 0 at the end of 17 hours.

Sugars Paired with Each Other

In all of the foregoing choice experiments the test sugar was paired with water. In the following experiments sugars were paired with other sugars at many different concentrations. The results are summarized in Table III. From a perusal of these data it may be seen that the results are in general agreement with what might have been expected from an examination of Figure 2. For example, it might have been predicted from Figure 2 that more of 1.0 *M* mannose than of 1.0 *M* fucose would be ingested because the curve for fucose is displaced to the left relative to the mannose curve. The prediction was verified when the two solutions were actually paired (Table III). Similarly, the relative volumes imbibed in other two-choice tests are in general agreement with the basic preference-aversion curves. On the other hand, the *absolute* volumes are not the same in the two types of experiments. Such a discrepancy is to be expected, because volume intake is dependent not only on the concentration of the test solution but on the concentration and identity of all other compounds to which the insect is simultaneously exposed. The total situation is the determinant. For example, it had previously been found by Dethier and Rhoades that the less preferred of two sugars in a paired test was treated as though it were water regardless of how much of it might have been ingested when it was presented alone. In every case here, with the exceptions of 1 *M* mannose paired with 1 *M* sorbose and 0.5 *M* mannose paired with 0.5 *M* sorbose, the same is true. The less preferred member of the pair is ingested at approximately the same level as water (*cf.* Tables I and III). Consequently, the sum of the two volumes ingested in a paired test is generally less than the sum of volumes of each sugar which would have been ingested when paired with water, unless, of course, the less preferred is

TABLE III

Volumes (ml./fly/24 hrs.) ingested when different sugars are paired (preferred sugar underlined)

No.	Solutions paired		Significance at 1% level
1	1.0*M* mannose <u>0.0077</u>	vs. 1.0*M* fucose 0.0007	+
2	1.0*M* mannose <u>0.0112</u>	vs. 1.0*M* sorbose 0.0054	+
3	1.0*M* mannose <u>0.0121</u>	vs. 0.1*M* fucose 0.0045	+
4	1.0*M* mannose <u>0.0154</u>	vs. 0.1*M* sorbose 0.0014	+
5	0.5*M* mannose <u>0.0113</u>	vs. 0.5*M* sorbose 0.008	+
6	0.1*M* mannose 0.0017	vs. 0.1*M* fucose <u>0.0138</u>	+
7	0.1*M* mannose 0.0065	vs. 0.1*M* sorbose 0.0084	−
8	0.01*M* mannose <u>0.0074</u>	vs. 0.0001*M* fucose 0.0046	+
9	0.001*M* mannose 0.0048	vs. 0.0001*M* fucose 0.0059	−
10	0.1*M* fucose <u>0.0129</u>	vs. 0.1*M* sorbose 0.0026	+
11	1.0*M* fucose 0.0000	vs. 1.0*M* sorbose <u>0.0034</u>	+
12	0.01*M* fucose <u>0.00294</u>	vs. 0.01*M* sorbose 0.00140	+
13	0.1*M* D-arabinose <u>0.0143</u>	vs. 0.1*M* L-arabinose 0.0043	+

being tested at a concentration at which it is not normally consumed more readily than water. In this last case the total consumption in the paired test would equal the sum of the two sugars tested individually.

In previous pairing of sucrose with glucose and sucrose with sucrose the volume intake of the preferred member was greater than in sugar-water pairs when the concentration in question fell at the peak of the preference-aversion curve, less if it fell on the ascending limb (*i.e.*, low concentrations) of the curve, and equal if on the

descending limb. In the tests reported here the volume intake of the preferred sugar in a pair generally equalled its intake when paired with water when the concentration in question fell at the peak of the preference-aversion curve.

Both sets of data (Tables I and III) suggest very strongly that volume intake is under sensory control, that is, that the stimulating effectiveness of a solution determines how much of it will be imbibed. Several aspects of the two-choice data underline the importance of the sensory rather than the nutritional characteristic of the sugar in regulating volume intake. Line 6 of Table III indicates a preference for 0.1 *M* fucose (non-nutritional) over 0.1 *M* mannose (nutritional). This result clearly indicates the choice of a stimulating sugar over a poorly stimulating one. The choice of 0.1 *M* fucose over 0.1 *M* sorbose (line 10), both sugars being non-nutritional, reflects the superior stimulating effectiveness of fucose at this level of concentration. The relative intake of two sugars at concentrations represented on the ascending limbs of the preference-aversion curves appears to be sense-controlled, the more stimulating sugar always being preferred (lines 4, 5, 6, 8, 10, 12). This conclusion is in agreement with the findings of Dethier and Rhoades (1954) relative to the intake of glucose and sucrose.

When comparisons are made which involve concentrations on the descending limbs of the preference-aversion curves, stimulating effectiveness alone is apparently no longer the sole controlling factor; hence, comparisons at these levels are more complex (lines 1, 11). For example, the preference for 1.0 *M* mannose over 1.0 *M* fucose (line 1) does not result simply from the superior stimulating effectiveness of mannose, for indeed fucose is the more stimulating; instead, the preference undoubtedly reflects a negative factor causing the decline in fucose intake (*cf.* Fig. 3) as being responsible for the preference of mannose in the two-choice situation.

Role of Sensory Systems

The foregoing results clearly implicate the sensory systems. There are three chemosensory systems (exclusive of olfaction) definitely known to be involved in the feeding behavior of *Phormia;* namely, the tarsal chemoreceptors, the labellar hairs, and the interpseudotracheal papillae (Dethier, 1955). The first two mentioned have been studied to a greater extent than the papillae, and most of the remarks regarding stimulating effectiveness in the foregoing section have been based on information so derived. However, on the basis of these studies alone mannose should not be imbibed at all, and certainly its preference-aversion curve should not fall between that of fucose and sorbose.

The difficulty was resolved by the discovery that mannose, while poorly stimulating to tarsi and labellum, was an effective stimulus for the papillae. Its effectiveness at this site explains quite satisfactorily other difficulties encountered in the foregoing section. Mannose is obviously accepted at high concentrations in preference to sorbose, and in preference to water because of its stimulating effect on the papillae. Even though it does not stimulate the tarsal and labellar hairs, except at very high concentrations, it gains access to the papillae as a result of the fly's extending and probing with its proboscis in its normal exploratory behavior and in the course of ingesting to satisfy its need for water.

The discovery of the stimulating effectiveness of mannose on the papillae led to a series of tests in which other selected sugars were applied to the three chemosen-

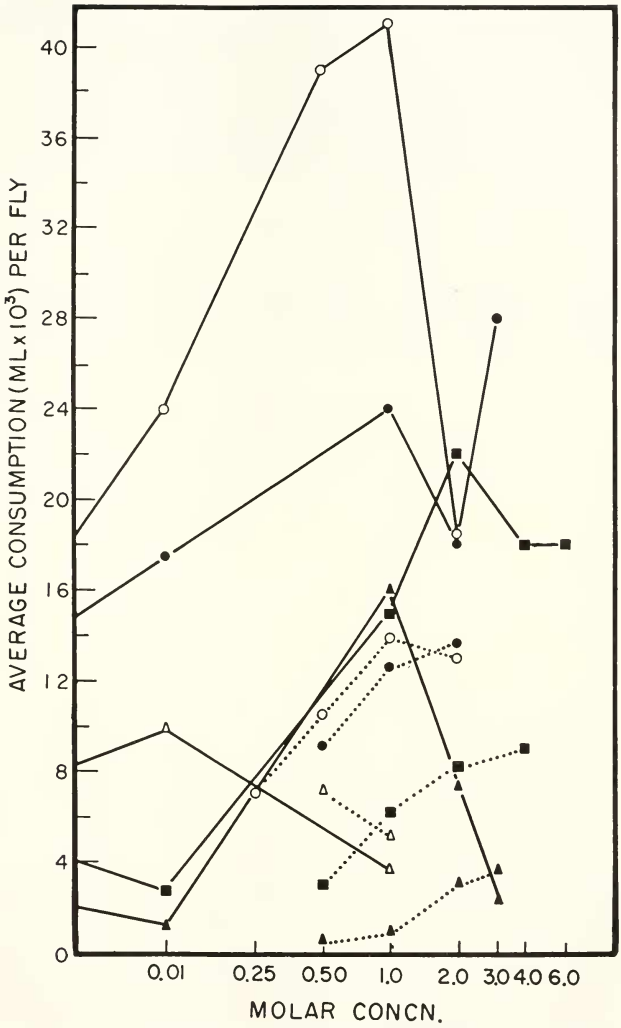

FIGURE 3. Comparison of ingestion measured by single feeding and by preference-aversion intake during the first twenty-four hours. Solid line, preference-aversion; dotted line, single feeding. ○ = sucrose, ● = glucose, ■ = mannose, △ = fucose, ▲ = sorbose.

sory systems. The results are given in Table IV. The most surprising result concerned L-arabinose, which was found to act as a repellent to the papillae even though it is acceptable in terms of its effect on tarsal and labellar hairs. This characteristic of L-arabinose was most unexpected. Clearly it stimulates the tarsal and labellar hairs, as a result of which the fly is moved to extend its proboscis and commence feeding. However, as soon as the solution comes into contact with the papillae, ingestion ceases abruptly. D-arabinose, by contrast, is acceptable to all three chemosensory systems and is consumed in appreciable quantities even though it is not utilized (Table I).

Relation Between Intake and Nutritional Value

From experiments in which different sugars were paired there were already indications that the stimulating rather than the nutritional characteristic of a sugar played a major role in regulating volume intake (*cf.* line 6 of Table III). The minor importance of nutritional factors, at least under experimental conditions, is

Table IV

Effectiveness of selected sugars in stimulating the three chemoreceptive systems of Phormia

Sugar	Tarsal threshold (molar)	Labellar hairs	Interpseudotracheal papillae
fucose	0.087	+	+
sorbose	0.140	+	+
mannose	7.59	−	+
D-arabinose	0.144	+	+
L-arabinose	0.536	+	R
D-xylose	0.440	+	
L-xylose	0.337	+	−
rhamnose	−	−	−
ribose	−	−	
lactose	−	−	−

(+ stimulating, − non-stimulating in all concentrations, R rejected)

revealed further by comparisons of the results of preference tests with sugar mixtures and the capacity of these mixtures for sustaining life. Two examples serve to illustrate the point, the behavior of flies with respect to glucose and D-arabinose and with respect to glucose and rhamnose.

Glucose alone at a concentration of 0.1 *M* supported life for 14 days (50% mortality); D-arabinose, for 3.5 days; a mixture containing 0.1 *M* glucose and 0.1 *M* D-arabinose, for 5.5 days. Survival on water alone averages three days (*cf.* also Hassett, Dethier and Gans, 1950). Yet in preference tests where glucose was paired with the non-nutritional mixture, flies consumed greater quantities of the mixture. Similarly, a mixture of 0.1 *M* glucose and 0.1 *M* rhamnose, which supported life for 8 days as compared to 3.5 days for rhamnose alone and 14 days for glucose alone, was consumed in greater quantity than glucose alone in a paired test (Table VII). Rhamnose paired with water was preferred slightly (Table I).

From these results it would appear that choices were made solely on the basis of the stimulating effect. There is no indication that either D-arabinose or rham-

nose is repellent, since each is in fact preferred to water. While neither interferes with the stimulating effect of glucose on sense organs (Table V), both either are toxic or block glucose utilization.

Intake of Mixtures of Sugars

Sometimes the acceptability of compounds of very low stimulating power cannot be demonstrated in a two-choice test with water or by simple acceptance threshold determinations. Accordingly, the ruse has frequently been employed of mixing two sugars in order to detect suspected additive or repellent properties. Kunze (1927) and von Frisch (1935), for example, found that sugars which were acceptable to the honeybee were strictly additive. Unfortunately the technique is deceptively simple, and the results cannot always be relied upon to give the desired sensory information because of the occurrence of two phenomena which have not been given due consideration. These two are synergism and inhibition. They can be demonstrated most easily and convincingly by measuring tarsal acceptance thresholds to sugars and sugar mixtures. They also occur at labellar hairs. Tests for inhibition and synergism have not been made with interpseudotracheal papillae,

Table V

Examples of inhibition revealed by ascertaining the effects of sugar mixtures on tarsal thresholds

Sugar	Effect	Sugar affected
mannose	inhibits	fructose
	does not affect	glucose, sucrose, fucose, maltose
sorbose	inhibits	glucose, fructose
fucose	does not affect	glucose, fructose
rhamnose	does not affect	fucose, glucose
	inhibits	fructose
D-arabinose	does not affect	glucose
mannitol	does not affect	fructose

but the occurrence of the phenomena at other sites indicates that an additive effect of sugars cannot be assumed as a matter of course.

For example, the median acceptance threshold for fructose is 0.0058; for glucose, 0.132; for an equimolar mixture of the two, 0.0078. In other words, the concentration at which the mixture is stimulating represents 0.0039 *M* glucose and 0.0039 *M* fructose. Even were the two sugars simply additive, they would not be expected to stimulate at this level. The fact that they do stimulate implies synergism. Mannose, on the contrary, when added to fructose inhibits it, that is, causes a ten-fold rise in the fructose threshold. This effect is not due to repellence because, for *Phormia,* mannose is preferred to water in all concentrations above threshold. Furthermore, mannose has no effect on such sugars as glucose, sucrose, or maltose.

The results of threshold tests with other mixtures are summarized in Table V. That the effects observed represent inhibition rather than repellence is further confirmed by the action of sorbose. Sorbose is stimulating in its own right, yet it causes an increase in the thresholds of glucose and fructose when mixed with them. Its action is revealed clearly in the following representative results (Table VI) where the per cent response of a sample of flies to various concentrations of glucose and of sorbose is compared to their response to a series of solutions which contain

0.5 moles of sorbose. In this case the stimulating effect of the mixture at low glucose concentrations stems entirely from the sorbose which is present. At higher concentrations of glucose, when the same amount of sorbose is present, there is little change in the stimulating effectiveness. Not only do the two sugars fail to add, but the stimulating effect to be expected of the high concentrations of glucose is absent. When, therefore, a smaller volume of a mixture of sugars is ingested than of either of the constituents alone, the result cannot always be ascribed to repellence, especially when both constituents can be shown in other tests to be preferred to water.

Galun (1955) has reported that all of the following sugars are repellent to *Musca domestica:* D-xylose, L-arabinose, ribose, rhamnose, and sorbose. This conclusion is based, however, on the fact that the addition of any of these to an acceptable sugar causes a lowering of intake. Unless the sugars can be shown to have a repellent effect when compared with water, the possibility of inhibition cannot be overlooked.

In the present studies some of the results of preference tests with sugar mixtures can be understood in terms of inhibition. For example, the volume intake of a mix-

TABLE VI

Effect of sorbose on glucose threshold

Molar concen. of glucose solutions	0.0625	0.125	0.25	0.50	1.0
Per cent response	0	5	15	50	80
Molar concen. of glucose solutions containing 0.5 *M* sorbose	0.0625	0.125	0.25	0.5	1.0
Per cent response	45	50	60	50	65
Molar concen. of sorbose solutions	0.0625	0.125	0.25	0.50	1.0
Per cent response	5	20	45	60	80

ture of mannose and glucose is greater than that of glucose alone, as would be expected (Table VII). In contrast, the intake of a mannose-fructose mixture, as compared with fructose alone, is not so great as would naturally be expected. Similarly with mixtures containing rhamnose there is a large increase in volume intake where the other sugar is glucose but no appreciable increase where the other sugar is fructose or sucrose (Table VII). This finding is in agreement with the threshold data (Table V) which indicate that rhamnose inhibits fructose but not glucose.

The situation with regard to sorbose is not so clear although there is a tendency for the intake of sorbose mixtures to be less than expected on a purely additive basis. Such a result would agree with the postulated inhibitory effect of sorbose on glucose and fructose. It must nevertheless be emphasized that the effect of sugar mixtures on the papillae is not known, so that the results obtained in preference tests of mixtures cannot be fully interpreted in terms of demonstrated inhibition at tarsal and labellar sites alone.

The difference between repellence and inhibition, at least with *Phormia,* is a real one. Since the tarsal and labellar hairs of *Phormia* have been shown to con-

sist of two receptors, one of which mediates rejection and one of which mediates acceptance (Dethier, 1955), a compound which is repellent might be expected to stimulate the rejection receptor while a compound which is an inhibitor might be expected to prevent stimulation of the acceptance receptor by interfering with the action of a stimulating compound on that receptor.

The only comparable study of mixtures on another insect is that of Wykes (1952), who measured ingestion of single sugars and mixtures of sugars by the honeybee. Although not explicitly stated, the experiment tested the hypothesis that the volume ingested of the four sugars examined, singly and in mixtures, was related to concentration by the formula $V = a + C$ where V is volume ingested at concentration C. For all four sugars, then, there was assumed to be a linear relationship between volume ingested and concentration, with a slope of unity and an intercept depending upon the sugar involved. Since, however, the units of volume

TABLE VII

Comparison of intake of mixed solutions with that of water or single sugars in a two-choice test

Concentration of each sugar in mixture	Vol. consumed ml./fly/24 hrs.	Water or sugar	Vol. consumed mL/fly/24 hrs.
0.05 *M* fucose and 0.05 *M* sorbose	0.0125	water	0.0019
0.5 *M* fucose and 0.5 *M* sorbose	0.0116	water	0.0030
0.05 *M* fucose and 0.05 *M* mannose	0.0213	water	0.0025
0.5 *M* mannose and 0.5 *M* sorbose	0.0184	water	0.0018
0.1 *M* fructose and 0.1 *M* mannose	0.0234	0.1 *M* fructose	0.0130
0.05 *M* glucose and 0.05 *M* mannose	0.0090	0.1 *M* glucose	0.0030
0.1 *M* glucose and 0.1 *M* mannose	0.0228	0.1 *M* glucose	0.0090
0.05 *M* glucose and 0.05 *M* sorbose	0.0099	0.1 *M* glucose	0.0162
0.05 *M* fructose and 0.05 *M* sorbose	0.0220	0.05 *M* fructose	0.0160
0.05 *M* glucose and 0.05 *M* rhamnose	0.0260	0.05 *M* glucose	0.0130
0.1 *M* glucose and 0.1 *M* rhamnose	0.0160	0.1 *M* glucose	0.0070
0.05 *M* fructose and 0.05 *M* rhamnose	0.0120	0.05 *M* fructose	0.0130
0.1 *M* fructose and 0.1 *M* rhamnose	0.0170	0.1 *M* fructose	0.0140
0.05 *M* sucrose and 0.05 *M* rhamnose	0.0180	0.05 *M* sucrose	0.0230
0.1 *M* glucose and 0.1 *M* D-arabinose	0.0210	0.1 *M* glucose	0.0080
0.05 *M* glucose and 0.05 *M* D-arabinose	0.0130	0.1 *M* glucose	0.0160

employed were arbitrary and apparently were changed from one concentration to the next, this hypothesis was not tested directly; it was implicitly assumed in the analysis of ingestion of mixtures. The experiments on mixtures consisted of measuring the volume ingested of a solution containing equal proportions by weight of two to four sugars with a total sugar concentration of $x\%$ and testing the significance of the difference between this value and the average of the volumes ingested of each of the component sugars at $x\%$. For example, the volume ingested of a solution containing 8.5% sucrose and 8.5% glucose was compared with half the sum of the volumes ingested of 17% glucose and 17% sucrose. It was found, rather surprisingly, that the calculated and measured figures were not significantly different; hence, volume and concentration are linearly related, with a slope of unity for sucrose and glucose within the concentration range 17.1 to 51.3%. Similar experiments indicate that the same relationship is true for maltose. Furthermore, with one ex-

ception, these sugars in the mixtures tested are neatly additive in their effect on ingestion. The one exception was the glucose-sucrose-fructose mixture, of which more was ingested than was predicted (*i.e.*, there was synergism). This may reflect the synergism noted above on the tarsal threshold of *Phormia* for a mixture of glucose and fructose.

The data for *Phormia* relating volume and concentration, whether for intake at a single feeding or preference-aversion experiments, never present so simple a picture as Wykes's results. The only similarity may be the striking parallelism (with the exception of fucose) of volume increase from low to the optimum concentrations on a semi-log plot of ingestion at a single feeding (Fig. 3). Preference-aversion experiments on ingestion of mixtures probably are not comparable to ingestion as measured by Wykes; clearly, in the former case simple additivity of sugars in a mixture is not the rule.

The Feeding Reaction

Initiation of feeding. From the foregoing experimental facts and all other available information one can reconstruct, at least in part, the behavior pattern of the normal feeding reaction insofar as it is now known.

The normal pattern consists essentially of extension of the proboscis, spreading of the labellar lobes, sucking, and regurgitation. Apparently any one of three factors may initiate proboscis extension: (1) olfactory stimuli operating primarily through the antennae; (2) taste and possibly tactile stimuli operating through the tarsal receptors; (3) internal factors causing extension spontaneously. In the presence of vapors of an attractive nature a fly will extend its proboscis (*cf.* also Minnich, 1921). If the antennae are amputated, this faculty is impaired. Water (if a fly is thirsty) or specific carbohydrates can stimulate the tarsi with a resultant proboscis extension. In the absence of any specific external stimuli the fly will frequently repeatedly extend its proboscis in an exploratory manner.

The proboscis having been extended in response to any one or combination of these clues, the first parts which come into contact with the substrate are the long hairs of the aboral labellar surface. If the stimulus now received is favorable, the labellar lobes are opened, thus presenting the oral surface to the food. Sucking then commences. The labellar hairs, therefore, can regulate spreading of the lobes and sucking. They can also regulate extension, although under natural conditions it must be quite unusual for the hairs of the retracted proboscis to be stimulated. It could well be that in the event of the omission of an initial step in the normal sequence of stimulation, *e.g.*, stimulation of the labellar hairs before the proboscis is extended, the hairs trigger the missing step, in this case extension, before initiating the remaining steps. Control of the hairs over sucking is easily demonstrated. If, in a fastened fly, a drop of liquid just at the threshold of rejection is placed on the open labellum, it remains undisturbed, and the fly regurgitates into it. Surface tension prevents the fly from closing the labellum, and the feet cannot be employed to remove the drop because they are fastened. If now a single labellar hair is stimulated with a concentrated sugar solution (*e.g.*, 1 *M* sucrose), the drop, diluted with regurgitated fluids, is immediately swallowed.

Having opened the labellar lobes and commenced swallowing, the fly would no longer be in complete sensory control of the situation were it not for the interpseudotracheal papillae. Once the labellar lobes are opened the majority of the aboral

hairs are no longer in contact with the solution. Even if they had been, the speed with which they adapt would certainly prevent a continual input from sugar stimulation from reaching the central nervous system. There is ample evidence that the papillae supply this defect.

Feeding can be monitored at four levels. If an odorous component of food attains a repellent level of concentration, feeding may be inhibited although ordinarily feeding will not have commenced under these conditions. Secondly, if the tarsal receptors are stimulated by unacceptable compounds, feeding is ordinarily stopped and the proboscis withdrawn. This reaction is, of course, the basis of all measurements of tarsal rejection thresholds. Thirdly, if the labellar hairs are affected by adverse stimuli, feeding stops. Fourthly, if the papillae are stimulated by unacceptable compounds, feeding is terminated.

As might be expected, these various levels of control are finely balanced. The coördination of sensory input from all of the receptor systems involved is extremely important for the proper accomplishment of feeding. Consider, for example, the relation between tarsal receptors and those on the mouthparts. Normally a fly will not commence feeding on a solution which has first been rejected by the tarsi. However, if arrangements are made to stimulate tarsi and mouthparts simultaneously with different solutions, the tightness of control of each system over feeding can be assessed. Application of sugar, however concentrated, on the tarsi will not cause feeding if a critical concentration of NaCl is placed on the labellum; but a low concentration of NaCl can be found which will be imbibed when the tarsi are stimulated with sugar, even though this salt is refused in the absence of tarsal stimulation. Conversely, concentrated NaCl on the tarsi will not prevent imbibition of sucrose applied to the labellum. The mouthparts, as might be expected, exert a tighter control.

On the mouthparts themselves the actions of the labellar hairs and interpseudotracheal papillae are usually coördinated. Experimentally either can be stimulated alone. The papillae alone are stimulated by inserting a micropipette between the closed labellar lobes or by rendering the hairs inoperative through waxing. The papillae are extremely sensitive to NaCl, and the application of salt by pipette causes an immediate cessation of feeding. However, it is sometimes possible to force salt imbibition by simultaneous stimulation of labellar hairs with concentrated sucrose. Swallowing is accomplished with great hesitation on the part of the fly if the salt solution is at all concentrated. Conversely, if the hairs are stimulated with NaCl while the papillae are stimulated with sucrose, feeding can be stopped, albeit somewhat slowly and temporarily. From the results of these two experiments it would appear that the papillae exercise tighter control over actual feeding than do the labellar hairs. The behavior of the fly toward L-arabinose confirms this. The hierarchy of command over sucking in ascending order is tarsi, labellar hairs, interpseudotracheal papillae. For proboscis extension and spreading of the labellar lobes, it is tarsi, labellar hairs. Stimulation of the papillae seldom causes proboscis extension or spreading of the lobes so that by means of a micropipette a fly can be induced to feed without extending its proboscis or expanding the labellum. In every case mentioned above the relative concentrations of the opposing stimuli are extremely critical insofar as the nature of the final response is concerned.

Control of volume intake. Although the various chemoreceptors generally work in harmony to regulate the economy of feeding response, the imbibition of liquids is

only the beginning of a longer and more complex chain of events. Once the insect has begun to feed, it obviously does not continue indefinitely. Assuming that the substance being eaten or drunk is an acceptable one and that its stimulating effect (odor or taste) initiated feeding, what are the factors which ensure continuance of feeding and control of volume intake? It seems unlikely that the initial stimulation is alone sufficient to supply momentum for continued feeding without itself continuing, or, in other words, that feeding once started continues automatically until shut off. It is more probable that there is an additional factor which drives continuous feeding and another which terminates it.

Odorous foods not only can supply the initial stimulus but can also continue to stimulate for the duration of feeding. With odorless foods such as sugars, uninterrupted stimulation is also possible. If the fly is standing in sugar, the tarsal receptors can supply a continuous sensory input to the central nervous system until they become adapted. The principal stimulation from the mouthparts during feeding originates at the interpseudotracheal papillae because most of the labellar hairs are no longer in contact with the solution once the lobes have been spread. Even if the labellar hairs were in contact with the sugar, they adapt very rapidly. An experiment can be designed to show that, in the absence of any stimulation except that from the labellar hairs, complete adaptation of these hairs brings an end to feeding. For example, a fly which is not thirsty can be made to drink water if one or more of the labellar hairs are stimulated with sugar. Adaptation of the hair or hairs being stimulated causes feeding to cease, whereupon stimulation of different hairs which are still sensitive results in resumption of swallowing. From this result it would appear that a continual sensory input is indeed essential to uninterrupted feeding. Even stimulation of the tarsal receptors can drive feeding, and one way to force flies to imbibe non-stimulating fluids (*i.e.*, those which are neither acceptable nor repellent) is to apply sucrose to the legs. For many of the insects in which feeding reactions have been studied the prerequisite of sensory input is the rule (*cf.* Dethier, 1953).

Under natural circumstances a fly does not feed to full capacity upon first contact with an acceptable food but rather takes repeated samples. This behavior is graphically demonstrated by automatic recording (Fig. 1). In this way each new extension of the proboscis places the labellar hairs again in contact with the solution for fresh stimulation which imparts renewed impetus to feeding. At some point in the proceedings, however, feeding finally ceases; a definite quantity has been consumed. This volume is not constant but depends upon the hunger state of the fly, the nature of the food, and its concentration. Clearly neither gut capacity nor carbohydrate requirements immediately controls volume intake (*cf.* also Dethier and Rhoades, 1954). Thus, under normal conditions intake may cease long before the gut is fully extended. Furthermore, an isolated head does not drink equal amounts of all sugars. It takes in, for example, less sorbose than fucose and less fucose than sucrose, indicating control by structures of head alone.

An explanation which conforms most closely to the facts as now known is that intake is shut off by sensory adaptation. As an examination of Table II will reveal, the rate of imbibition and the duration of feeding increase with increasing concentration—up to a point. Since rate does increase with concentration and since maximum rates for different sugars are greatest for the more stimulating ones,

there is reason to conclude that *rate* of intake is related to sensory input. It is highly probable, therefore, that the relationship prevails over the entire concentration range but that at a certain point (where measured rate declines) some negative factor intervenes. Since gram intake never declines nor becomes constant, the negative factor cannot be the amount of sugar or excessive or repellent stimulation. The cause must be sought in some other characteristic of the solutions. Increase in viscosity at high concentrations is one limiting factor. Measurements of rates of intake of a series of glycerol solutions of 1 *M* sucrose showed that rate decreases sharply with relatively small increases in viscosity. This finding is in agreement with the results which Betts (1929) had obtained in experiments with honeybees, where rate of intake declined sharply as concentrations of sugar exceeded 50% by weight. Betts concluded that viscosity was the limiting factor in this concentration range. At lower concentrations, however, she observed little change in rate with change in either viscosity or concentration. For the honeybee, temperature appears to exercise greater control over rate of intake than concentration does.

From the fact that duration of feeding increases with concentration one may infer that adaptation is one factor bringing an end to feeding. This inference is in accord with observed increases in adaptation time with increased concentration (Dethier, 1952). Additional evidence in support of this view derives from the observation that a fly which has ceased to feed on a given concentration may be induced to continue on a higher one and that a fly which has been feeding on a high concentration refuses to continue feeding on a lower one. In this respect isolated heads behave similarly. If the inference is correct, it would appear that flies adapt most quickly to fucose and less quickly to mannose, glucose, and sucrose, respectively, because this is the inverse order of duration of feeding.

Although the immediate cessation of imbibition can be explained in terms of adaptation, peripheral and central, and there is no evidence of action by internal factors at this point, it is almost certain that subsequent intake at various times after feeding to repletion is regulated by internal factors. These factors have been investigated and will be discussed in a latter communication.

Summary

1. The ingestion of sucrose, glucose, fucose, sorbose, mannose, and lactose by the blowfly *Phormia regina* was studied by means of preference-aversion tests conducted for four-day periods; individual feeding tests; measurements of the sensitivity of the different chemoreceptor systems; measurements of volume intake of mixed solutions; and longevity tests.

2. The preference-aversion curves for all sugars studied indicated an increase in volume intake with increasing concentration up to an optimum point, after which there was a decrease in intake. At very low concentrations water was preferred to sugar.

3. Volume intake measured by individual feeding tests did not exhibit a pronounced decline at high concentrations. The difference between this finding and the one noted above resulted from the fact that flies ingested a maximum volume of concentrated solutions during the first visits to the pipette and then gradually ceased feeding altogether, while their ingestion of less concentrated solutions con-

tinued repeatedly over the entire test period. In all experiments the weight of sugar taken increased over the entire concentration range.

4. There is no relation between the amount of sugar taken and its nutritive value.

5. Volume intake is under sensory control. The coördinated actions of three principal chemosensory systems regulate the complete feeding reaction. The intake of mixed solutions depends upon the stimulating effectiveness of the mixture and whether or not any of the components exhibit synergism or inhibition. Some sugars show inhibition but no repellence.

6. The initiation of the feeding reaction is under sensory control. Continuance of feeding is dependent upon continuous sensory input. The rate of imbibition increases with concentration until viscosity begins to exert a restraining effect. The termination of feeding may be brought about by adaptation.

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